

Detection of *Cryptosporidium felis* and *Giardia duodenalis* Assemblage F in a cat colony

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Received 8 December 2005; received in revised form 9 March 2006; accepted 9 March 2006

Abstract

Eighteen cats, 3–6 months of age, bred and housed in a closed colony, were transferred from that colony and placed in separate stainless steel cages in a building designed for housing animals. At daily intervals, feces were collected from the litter pans in each cage, pans and cages were cleaned, and fresh food and water were provided. Beginning 4 weeks after the transfer, oocysts of *Cryptosporidium* were detected in the feces of two cats by brightfield microscopy. For the following 21 days, with minor exceptions, feces from each cat were collected daily and examined by immunofluorescence microscopy and by molecular methods that included DNA extraction, 18S rDNA gene amplification, and DNA sequence analysis. Within those 22 days, every cat was found to be infected with *Cryptosporidium felis* and excreted oocysts for 6–18 days. Eight of these 18 cats also excreted cysts of *Giardia duodenalis* Assemblage F, a genotype found only in cats. Six *Giardia* infections were concurrent during part of the patency with *C. felis* infections. Neither diarrhea nor other signs of illness were observed in any of the cats during this time. Because *C. felis* is zoonotic these findings suggest that care should be taken by veterinary health care providers and others in close contact with cats, even when cats appear healthy and asymptomatic.

Published by Elsevier B.V.

Keywords: *Cryptosporidium felis*; Feline; Cat; Prevalence; Genotyping; Zoonoses

1. Introduction

In the first report of cryptosporidiosis in cats Iseki (1979) described oocysts found in feces, light and electron microscopic observations of endogenous

stages, and attempts to transmit the organism to other host species. He named the organism *Cryptosporidium felis*. A widespread zoonotic species capable of infecting many species of animal hosts, *Cryptosporidium parvum*, has also been reported to infect cats (Lappin et al., 1997b; Keith et al., 2003; Scorza et al., 2003). There are additional reports of cats infected with unidentified species of *Cryptosporidium* in countries worldwide including Japan (Uga et al.,

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1989; Arai et al., 1990), the United States (Poonacha and Pippin, 1982; Koch et al., 1983; Monticello et al., 1987; Nutter et al., 2004), Germany (Augustin-Bichl et al., 1984), Czechoslovakia (Paulasek, 1985), the United Kingdom (Bennett et al., 1985), Switzerland (Egger et al., 1990), and Australia (McGlade et al., 2003a). A third species, *Cryptosporidium curyi*, with oocysts averaging $32\ \mu\text{m} \times 31\ \mu\text{m}$, was reported in cats in Brazil (Ogassawara and Benassi, 1986). This organism, more than six times larger than all other *Cryptosporidium* spp. is not considered a valid species.

When oocysts of *Cryptosporidium* have not been clearly identified by molecular methods it is not possible to accurately determine their species or their role in disease. Cats have been implicated as sources of cryptosporidiosis both in humans with immunosuppressive diseases (Lewis et al., 1985; Koch et al., 1983; Bennett et al., 1985) and in immunocompetent persons (Edelman and Oldfield, 1988; Egger et al., 1990). However, in none of these cases was it known if the same species as found in cats initiated infection or caused disease in humans. In only one report, in which patients with cryptosporidiosis also had cats as pets, was *C. felis* diagnosed by molecular methods (Morgan et al., 2000).

Surveys have been conducted worldwide to determine the prevalence of *Cryptosporidium* infection in cats. Seroprevalence studies conducted in Scotland (Tzipori and Campbell, 1981; Mtambo et al., 1995), the United States (Lappin et al., 1997a; McReynolds et al., 1999), and the Czech Republic (Svobodova et al., 1994) to detect antibodies to *Cryptosporidium* in cats found that 8.3–87% of cats surveyed had been exposed to *Cryptosporidium* spp. Of the few coprology based surveys, oocyst measurements were sometimes provided but none used molecular methods to confirm the species of *Cryptosporidium*. Reports of other surveys that provided neither oocyst size nor molecular data included: Hyogo Prefecture, surrounding Kobe, Japan, where 3.9% of 507 cats committed to the Animal Administration Office were excreting oocysts (Uga et al., 1989); Brno, Czech Republic, where 5.9% of 135 cats brought to the university veterinary clinic were excreting oocysts (Svobodova et al., 1994); Surabaya, Indonesia, where 2.4% of 532 cats were excreting oocysts (Katsumata et al., 1998); and surveys in which commercially available ELISA test kits yielded questionable results (Hill et al., 2000; Spain et al.,

2001; Cirak and Bauer, 2004). Reports of surveys in which oocyst size was determined (Table 1) included: Tokyo Metropolitan Animal Protection Center, with 3.8% of 608 domestic cats infected (Arai et al., 1990); Glasgow, Scotland with 8.1% of 235 domestic and feral cats infected, mostly young and newborn kittens with asymptomatic infections (Mtambo et al., 1991); farms near Glasgow with 12.3% of 57 cats infected (Nash et al., 1993); and the Czech Republic with one cat infected (Hajdusek et al., 2004). In Perth, Australia 1.2% of 162 cats were infected and isolates from these cats were subjected to gene sequence analysis for the first time (Sargent et al., 1998). Analysis of the short bp rDNA was distinctly different from that of *C. parvum* and was referred to as the feline *Cryptosporidium* genotype. The same researchers found oocysts of the same size and 18S rDNA sequences in four other cats and referred to these findings as additional evidence for *C. felis* in cats (Morgan et al., 1998). In Portugal a cat was reported to be infected with *C. felis* (as differentiated from *C. parvum* genotypes) based on RFLP analysis with TRAP C-1 and COWP gene fragments (Alves et al., 2001).

The first report of the feline *Cryptosporidium* genotype, identical to that described by Sargent et al. (1998), in human fecal specimens was from HIV-infected patients (Pieniazek et al., 1999). There have now been 40 reported cases of human infection with *C. felis*, involving both immunocompromised and immunocompetent persons, confirmed by molecular methods. These have been identified in persons in France (Guyot et al., 2002), Italy (Caccio et al., 2002; Leoni et al., 2003), Portugal (Alves et al., 2001), the United Kingdom (Pedraza-Diaz et al., 2001), Switzerland (Morgan et al., 2000), Thailand (Gatei et al., 2002; Tiangtip and Jongwutiwes, 2002), Haiti (Guyot et al., 2001), Peru (Xiao et al., 2001; Cama et al., 2003), and the United States (Morgan et al., 2000).

An unidentified species of *Cryptosporidium* oocysts from a farm cat were fed to two neonatal lambs (Mtambo et al., 1996). The lambs excreted *Cryptosporidium* oocysts but they were not further identified. In contrast, *C. felis* infection was clearly identified in a cow based on oocyst measurements of $4.3 \pm 0.4\ \mu\text{m}$ and confirmed by SSU rRNA gene sequence data (Bornay-Llinares et al., 1999).

Giardia infection in cats was first described as *G. cati* Deschiens 1925 (Levine, 1973). *Giardia cati* was

Table 1

Cryptosporidium oocyst and molecular data reported from cats, humans, and a bovine host

Species name	Oocyst data (μm)	Molecular data	Source	Author
<i>C. felis</i>	5.0×4.5	N/A	Cat	Iseki (1979)
<i>Cryptosporidium</i> spp.	4.0–5.0 diameter	N/A	Cat	Uga et al. (1989)
<i>Cryptosporidium</i> spp.	4.3–4.7	N/A	Cat	Arai et al. (1990)
<i>Cryptosporidium</i> spp.	5.2×4.3	N/A	Cat	Mtambo et al. (1991)
<i>Cryptosporidium</i> spp.	5.0×4.5 6.0×5.0	N/A	Cat	Nash et al. (1993)
<i>Cryptosporidium</i> feline genotype	4.6×4.0	18S rDNA	Cat	Sargent et al. (1998)
<i>C. felis</i>	Same as Sargent	Same as Sargent	Cat	Morgan et al. (1998)
<i>C. felis</i>	N/A	TRAP C-1, COWP	Cat	Alves et al. (2001)
<i>Cryptosporidium</i> spp.	N/A	SSU rRNA	Cat	McGlade et al. (2003a)
<i>Cryptosporidium</i> spp.	4.0–5.0 diameter	N/A	Cat	Lent et al. (1993)
<i>C. felis</i>	$4.6 \pm 0.3 \times 4.2 \pm 0.2$	SSU rRNA, COWP	Cat	Hajdusek et al. (2004)
<i>C. felis</i>	4.6×4.2	SSU rRNA	Cat	Present report
<i>C. felis</i>	N/A	18S rDNA, HSP70	Human	Morgan et al. (2000)
<i>C. felis</i>	N/A	SSU rRNA RFLP, GP60	Human	Alves et al. (2001)
<i>C. felis</i>	N/A	18S rRNA	Human	Guyot et al. (2001)
<i>C. felis</i>	N/A	SSU rRNA RFLP	Human	Pedraza-Diaz et al. (2001)
<i>C. felis</i>	N/A	SSU rRNA RFLP	Human	Xiao et al. (2001)
<i>C. felis</i>	N/A	18S rRNA RFLP	Human	Gatei et al. (2002)
<i>C. felis</i>	$4.6 \pm 0.7 \times 4.1 \pm 0.6$	18S rRNA	Human	Tiangtip and Jongwutiwes (2002)
<i>C. felis</i>	4.5–4.9	SSU rRNA, COWP	Human	Caccio et al. (2002)
<i>C. felis</i>	N/A	RT-PCR for dsRNA	Human	Leoni et al. (2003)
<i>C. felis</i>	N/A	SSU rRNA RFLP	Human	Cama et al. (2003)
<i>C. felis</i>	$4.3\text{--}4.5 \pm 0.5$	SSU rRNA	Cow	Bornay-Llinares et al. (1999)

reclassified within the *G. duodenalis* species complex by Filice (1952) based on trophozoite morphology. With the advent of molecular tools, the *G. duodenalis* complex was divided into assemblages (genotypes), and cats were reported as hosts of Assemblages A and F (Thompson et al., 2000). A cross-transmission study pre-dating genotype identification demonstrated that when cats were experimentally exposed to *Giardia* of human origin, 25% or fewer became infected (Kirkpatrick and Green, 1985). This finding, combined with the lack of detection of Assemblage A in recent molecularly based prevalence studies (McGlade et al., 2003b; Itagaki et al., 2005; Lalle et al., 2005) suggests that cats are not a significant source of *G. duodenalis* Assemblage A. Although propagation of Assemblage F has been reported in suckling mice (Monis et al., 2003), there are no reports of natural infections with this Assemblage in non-feline hosts. Thus, this assemblage likely represents little or no risk to humans. Giardiasis has been reported in cats worldwide (Kirkpatrick and Farrell, 1984; Belosevic et al., 1984; Hill et al., 2000; Spain et al., 2001; McGlade et al., 2003b; Itagaki et al.,

2005; Lalle et al., 2005). However, clinical signs are rare, diarrhea being the most common sign (Barr and Bowman, 1994).

There have been no reports of outbreaks as such of cryptosporidiosis from *C. felis* or other *Cryptosporidium* spp. in cat colonies or other animal holding facilities such as animal shelters although *Cryptosporidium* spp. has been detected in a large number of kittens at two locations (Uga et al., 1989; Marks et al., 2004). The present study documents infection of eighteen 3- to 6-month-old cats with *C. felis* and *G. intestinalis* Assemblage F in a closed colony all within a 22-day period.

2. Material and methods

2.1. Sources and collection of specimens

Mixed breed domesticated cats (*Felis domestica*) were bred and weaned in a closed cat colony that was managed as described (Dubey, 1995). After weaning,

eighteen 3- to 6-month-old cats were placed in individual stainless steel cages in a separate room of a building designed to house laboratory animals and approved for use by the local animal care and use committee. Cats were examined daily for signs of illness. Cages and litter pans were cleaned daily. Fresh food and water were always available.

Beginning 4 weeks after cats were placed in separate cages oocysts were detected in feces of two cats by brightfield microscopy during a routine daily fecal examination of all cats. Except for a lapse on the following 3 days, feces were collected over the following 21 days from the litter pan in each cage. Feces were placed in a plastic specimen cup that was capped, labeled to identify the cat and date of collection, refrigerated, and transported to the Environmental Microbial Safety Laboratory for processing. Minor exceptions to collection days are noted in Table 2. To prevent contamination of specimens, technicians wore disposable gloves that were changed after each specimen was collected. Feces were stored in a dedicated refrigerator at 5 °C and processed within 1–3 days of collection.

2.2. Cleaning of specimens from feces

Feces were processed as previously described (Fayer et al., 2000). Fifteen grams of feces from each plastic specimen cup was transferred to a 50 ml centrifuge tube containing 35 ml dH₂O and mixed with the aid of a vortexer (Vortex-Genie, Scientific Industries, Bohemia, New York). After the fecal suspension was passed through a 45 µm pore size brass screen and collected in a second 50 ml tube, the final volume was adjusted to 50 ml with dH₂O. Tubes were centrifuged at 1800 × g for 15 min, the supernatant discarded, and the pellet suspended in 25 ml dH₂O and mixed with the aid of a vortexer. To each tube 25 ml of CsCl (1.4 g/l) was added, the suspension mixed, and the tubes centrifuged at 300 × g for 20 min. Four millilitres of supernatant was aspirated from the top of each suspension and transferred to a 15 ml centrifuge tube; dH₂O was added to reach a final volume of 15 ml. Tubes were capped and centrifuged at 1800 × g for 15 min, washed with dH₂O and centrifuged twice before the final pellet was resuspended in 500 µl of dH₂O. Portions of this suspension were examined by immunofluorescence microscopy and molecular analysis as described below.

2.3. Fluorescence microscopy examination

From each daily cat fecal specimen that had been cleaned and concentrated by CsCl density centrifugation, a 100 µl suspension was transferred to a microcentrifuge tube and pelleted. The pellet was resuspended in premixed Merifluor reagent (Meridian Diagnostics, Cincinnati, OH) and 2 µl of suspension was transferred to a glass microscope slide. The slide was covered with a glass coverslip and examined by fluorescence microscopy at 400× using a Zeiss Axioskop equipped with epifluorescence and an FITC-Texas Red™ dual wavelength filter.

2.4. Molecular analysis

After daily fecal specimens from each cat had been cleaned and concentrated by CsCl density centrifugation, DNA was extracted from a 50 µl suspension, using a DNeasyTissue Kit (Qiagen, Valencia, CA). To maximize recovery of DNA, the nucleic acid was eluted in 100 µl of AE buffer (elution buffer included in DNeasyTissue Kit).

For *Cryptosporidium*, a two-step nested PCR protocol was used to amplify the SSU rRNA gene. The SSU rRNA gene fragment was amplified by PCR previously described by Xiao et al. (1999). For the primary PCR step, the PCR mixture contained 1× PCR buffer, 3 mM MgCl₂, 0.2 mM each dNTP, 2.5 U Taq, 2.5 µl of BSA (0.1 g/10 ml), and 1 µM for each forward and reverse primer in a total of 50 µl reaction volume. A total of 35 cycles, each consisting of 94 °C for 45 s, 59 °C for 45 s, and 72 °C for 1 min, were performed; an initial hot start at 94 °C for 3 min and a final extension step at 72 °C for 7 min were also included. For the secondary PCR step, the PCR mixture was identical except that a concentration of 1.5 mM MgCl₂ was used. A total of 40 cycles, each consisting of 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 2 min, were performed; an initial hot start at 94 °C for 3 min and a final extension step at 72 °C for 7 min were also included.

For *Giardia*, A fragment of the SSU-rDNA (~292 bp) gene was amplified by PCR as previously described (Hopkins et al., 1997). For the primary PCR step, the PCR mixture contained 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM each dNTP, 2 U Taq, 2.5 µl of dimethyl sulfoxide (DMSO), and 0.5 µM for each forward and reverse primer in a total of 50 µl reaction

Table 2
Microscopic detection of *Cryptosporidium* (C) and *Giardia* (G) in cat feces

CAT ID	Days																	
	1	5	6	7	8	9	10	12	13	14	15	16	17	18	19	20	21	22
1	-	C	NE	C	C	C	C	C	C	C	NE	C	C	C	NE	NE	NE	NE
2	-	NE	-	-	-	-	-	C	-	NE	NE	NE	NE	NE	NE	NE	NE	NE
3	-	-	-	-	-	-	C	C	-	-	-	-	-	-	NE	NE	NE	NE
4	-	-	C	C	-	C	C	-	C	C	-	-	NE	NE	NE	NE	NE	NE
5	-	C	C	C	C	-	-	C	C	C	-	C	-	-	NE	NE	NE	NE
6	-	-	-	-	-	C	-	-	C	-	-	-	-	C	-	C	C	C
7	-	-	C	-	-	-	C	-	-	-	-	-	C	C	C	C	C	C
8	-	C	C	-	-	-	-	C	C	C	-	-	-	-	C	NE	C	C
9	C	C	C	C	-	-	C	C	-	C	-	-	-	-	NE	NE	NE	NE
10	C	C	C	C	C	-	C	C	-	C	-	-	-	-	NE	NE	NE	NE
11	-	C	C	C	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE
12	-	C	C	-	-	-	-	C	C	C	-	-	-	-	-	-	-	-
13	-	C	C	C	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE
14	-	G	C+G	C+G	C+G	C+G	C	C	C	C	G	G	G	G	NE	NE	NE	NE
15	-	C	C+G	C+G	G	C+G	C+G	C	C	C	G	C+G	-	G	NE	NE	NE	NE
16	-	C	-	C	C+G	C+G	C+G	C	C	C	G	-	G	C+G	NE	NE	NE	NE
17	-	C	NE	-	-	-	C	C	C	G	G	G	G	C+G	C+G	C+G	C+G	C+G
18	-	NE	NE	NE	NE	NE	NE	C	-	-	G	G	C+G	C+G	C+G	C+G	C+G	C

NE: feces were not examined because an insufficient quantity was collected or cats were no longer in the colony; (-) specimen negative for *Cryptosporidium* and *Giardia*. On days 2–4 and 11, feces were not collected.

volume. A total of 35 cycles, each consisting of 96 °C for 45 s, 58 °C for 30 s, and 72 °C for 45 s, were performed; an initial hot start at 96 °C for 2 min and a final extension step at 72 °C for 4 min were also included. For the secondary PCR step, the PCR mixture was identical. A total of 35 cycles, each consisting of 96 °C for 45 s, 58 °C for 30 s, and 72 °C for 45 s, were performed; an initial hot start at 96 °C for 2 min and a final extension step at 72 °C for 4 min were also included.

PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining.

Exonuclease I/shrimp alkaline phosphatase (Exo-SAP-IT™) (USB Corporation, Cleveland, OH) was used to purify the PCR products that were then sequenced using the same PCR primers in 10 µl reactions with Big Dye™ chemistries and an ABI3100 sequencer analyzer (Applied Biosystems, Foster City, CA). After each specimen was sequenced in both directions, chromatograms from each strand

were aligned and inspected using Lasergene software (DNASTAR Inc., Madison, WI).

3. Results

3.1. Clinical signs of infection

All cats were examined daily throughout the 22-day period. They appeared healthy and had no observable signs of illness. Food and water intake were normal. Feces were soft and formed, with no indication of diarrhea.

3.2. Brightfield and immunofluorescence microscopy

During the first 4 weeks after cats has been placed in individual cages, feces were subjected to sucrose

Table 3

Molecular detection of *Cryptosporidium* (C) and *Giardia* (G) in cat feces by polymerase chain reaction

CAT ID	Days															
	1	5	6	7	8	9	10	15	16	17	18	19	20	21	22	
1	NE	C	NE	C	C	C	C	NE	C	C	C	NE	NE	NE	NE	
2	NE	NE	-	C	-	-	-	NE	NE	NE	NE	NE	NE	NE	NE	
3	NE	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE	
4	NE	C	C	C	C	C	C	G	-	NE	NE	NE	NE	NE	NE	
5	NE	C	C	-	C	C	C	-	C	-	-	NE	NE	NE	NE	
6	NE	C	C	C	C	C	C	-	C	C	-	C	C	C	C	
7	NE	C	C	C	C	C	C	-	C	C	C	C	C	C	C	
8	NE	C	C	C	C	-	C	-	C	C	-	C	NE	C	C+G	
9	C	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE	
10	C	C	C	C	C	C	C	-	G	-	-	NE	NE	NE	NE	
11	NE	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE	
12	NE	C	C	C	C	C	C	-	-	-	-	C	C	C	C	
13	NE	C	C	C	C	C	C	-	-	-	-	NE	NE	NE	NE	
14	NE	C+G	C+G	C	C+G	C+G	C+G	G	G	G	G	NE	NE	NE	NE	
15	NE	C+G	G	C+G	C+G	C+G	C	C+G	C+G	C	G	NE	NE	NE	NE	
16	NE	C	C	C	C+G	C+G	C+G	G	G	G	G	NE	NE	NE	NE	
17	NE	C	C	-	-	C	-	G	C+G	C+G	C+G	C+G	C+G	C+G	C+G	
18	NE	NE	NE	NE	NE	NE	NE	G	G	C+G	C+G	C+G	C+G	C+G	C+G	

NE: feces were not examined because an insufficient quantity was collected or cats were no longer in the colony; (-) specimen negative for *Cryptosporidium* and *Giardia*. On days 2–4 and 11, feces were not collected; on days 12–14, an insufficient quantity of feces was collected for testing.

flotation and routinely examined for parasites by brightfield microscopy but neither *Cryptosporidium* nor *Giardia* were detected. At the end of this 4-week period oocysts were observed in the feces of two cats during routine examination of all cats. A period of 3 days lapsed until feces of all cats began to be examined by immunofluorescence microscopy and molecular methods. Days on which *Cryptosporidium* oocysts and *Giardia* cysts were found in cat feces are presented in Table 2. During the 22 days of observation all 18 cats in the colony excreted *Cryptosporidium* oocysts. Although specific quantitative data were not obtained, the greatest number of oocysts excreted per cat was observed on days 5 and 6 at which time 100 or more oocysts were observed per 400× microscopic field of feces from 6 cats. On all other positive days 10 or less oocysts were observed per microscopic slide.

Six cats excreted *Giardia* cysts, sometimes concurrently with oocyst excretion. For all specimens, 100 or fewer cysts were observed per 400 × microscopic field.

3.3. Molecular detection

Days on which PCR positive specimens were detected and the products subjected to gene sequence analysis are presented in Table 3. Within the 22-day period of examination all 18 cats were found to be infected with *C. felis* on 1 or more days. Sequence data for all cats had 100% homology with *Cryptosporidium felis* (GenBank accession number, AF112575). Over the 22-day period that cats were observed, specimens were not available from any of the cats for molecular analysis on days 2, 3, 4, 11, 12, 13, and 14. Six cats were found to be infected with *G. duodenalis*. Sequence data for all cats had 100% homology with *G. duodenalis* genotype F (GenBank accession number, AF199444).

4. Discussion

The present study documents infection of eighteen 3- to 6-month-old cats with *C. felis* and *G. intestinalis* Assemblage F in a closed cat colony within the brief period of 22 days. Two possibly similar situations of cryptosporidiosis in cat colonies have been reported. One appeared as a brief remark in a survey study (Uga

et al., 1989). This situation, cited as unpublished data, described a 2-year period at a university animal laboratory in Japan where cats were bred and indicated that the rate of cryptosporidiosis ranged from 10 to 40% (Uga et al., 1989). The other was a study designed to compare direct immunofluorescence microscopy with acid-fast staining and commercially available immunoassay detection kits (Marks et al., 2004). They reported that of 104 kittens examined 89% were found infected with *Cryptosporidium* spp., but it was not clear if this was an outbreak. Although all feces were collected at the same housing facility, it was not indicated how many kittens were present at the same time, how many kittens were found infected at a specific time, or if random kittens were examined over a long time period as they passed through the facility.

Overall, PCR was far more sensitive than immunofluorescence microscopy in detecting infections (Tables 2 and 3). For example, in the first 10 days when specimens were examined by both methods 92 versus 51 specimens were found positive for *Cryptosporidium* by PCR versus microscopy and only three specimens were positive by microscopy that were negative by PCR. The low numbers of oocysts in many specimens and the lower sensitivity of the immunofluorescence method could have accounted for this difference. The same number of *Giardia* positive specimens was found during this time by both methods, with positive findings differing among four specimens.

It is not known if the 3- to 6-month-old cats in the present study had been previously infected with either the same or other species of *Cryptosporidium* or *Giardia* before they were moved into individual cages. Cats in another facility were reported to be approximately the same age, 8- to 16-weeks-old, when they were found infected with *Cryptosporidium* spp. (Marks et al., 2004).

In the present study none of the cats appeared ill and none had diarrhea during the period when oocysts and cysts were detected. Marks et al. (2004) reported that the consistency of fecal specimens varied markedly but did not appear to be associated with the presence or absence of oocysts. Likewise, in cats experimentally inoculated with small *Cryptosporidium* oocysts from a naturally infected cat none of the experimentally infected cats showed clinical

symptoms (Asahi et al., 1991). In other cases, cats infected with *Cryptosporidium* had diarrhea. Some had concurrent neoplasia (Monticello et al., 1987; Goodwin and Barsanti, 1990; Lent et al., 1993) and others had no detectable pathogens or underlying disease (Poonacha and Pippin, 1982; Bennett et al., 1985; Lappin et al., 1997b). However, the species of *Cryptosporidium* was not clearly determined in any of these cases.

Except for the description of the life cycle of *C. felis* (Iseki, 1979) and the biological nature of the parasite (Asahi et al., 1991) all other publications of cryptosporidiosis in cats contain case reports, surveys, or testing of diagnostic methods.

In addition to the present report few reports of cryptosporidiosis in cats document both oocyst morphology and gene sequence analysis data for the same specimens. The first to do so found oocysts from cats averaging $4.6\ \mu\text{m} \times 4.0\ \mu\text{m}$ and provided sequence data for the 18S rDNA gene (Sargent et al., 1998). The genotype was unique among *Cryptosporidium* spp. and was termed the feline *Cryptosporidium* genotype (Sargent et al., 1998). In support of this finding additional cats were found with the same size oocysts and the same 18S rDNA gene sequence and these were said to be infected with *C. felis* (Morgan et al., 2000). Others that provided molecular data for *C. felis* reported oocysts from a cat averaging $4.6\ \mu\text{m} \times 4.2\ \mu\text{m}$ (Hajdusek et al., 2004), oocysts from humans averaging $4.6\ \mu\text{m} \times 4.1\ \mu\text{m}$ (Tiangtip and Jongwutiwes, 2002) and $4.5\text{--}4.9\ \mu\text{m}$ (Caccio et al., 2002), and oocysts from a cow averaging $4.3\text{--}4.5\ \mu\text{m}$ (Bornay-Llinares et al., 1999). The sizes of these oocysts and of those observed in the present outbreak are smaller than those established for *C. parvum* and for those in the original description of *C. felis* by Iseki.

Giardia spp. has been reported in cats from breeding colonies, animal shelters, boarding facilities, pet shops, and private owners (Belosevic et al., 1984; Hill et al., 2000; Spain et al., 2001; McGlade et al., 2003a,b).

Giardia duodenalis Assemblage F is not known to be zoonotic and therefore is not thought to be a public health concern. Because *C. felis* is known to be zoonotic the present findings suggest that care should be taken by veterinary health care providers and others in close contact with cats, even when cats appear healthy and asymptomatic.

Acknowledgements

The authors thank Oliver Kwok, Kristin Cameron, Brooke Reich, and Brandon Hall for technical services in support of this study.

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